Proposed claims: June 4, 1998 Our Ref. P-59-5

(1) A kit comprising:

a transparent non-porous or translucent non-porous system capable of retaining or containing a fluid or solution, which system comprises:

(i) a porous or non-porous substrate;

(ii) fluid or solution; and

(iii) a double-stranded oligonucleotide or polynucleotide comprising a nucleic acid of interest which is directly or indirectly fixed or immobilized to said substrate and one or more non-radioactive chemically-labeled nucleotide probes which are hybridized with said nucleic acid of interest, said nucleotide probes comprising one or more signaling moieties which are capable of generating a soluble signal,

wherein said kit is used to detect said nucleic acid of interest by said soluble signal which is generated by contacting said nucleotide probe with said fluid or soltion and which signal is dissolved and diffused in said fluid or solution in said non-porous system.

(2) A kit in accordance with claim 1 wherein said substrate is glass or other siliceous material.

(3) A kit in accordance with claim 1 wherein said chemically-labeled nucleotide is the compound,

[formula 1]

wherein B represents a purine, 7-deazapurine or pyrimidine moiety covalently bonded to the C$^{1'}$ -position of the sugar moiety, provided that when B is purine or 7-deazapurine, it is attached at the N^{9}-position of the purine or deazapurine, and when B is pyrimidine, it is attached at the N^{1} -position;

wherein A represents a moiety consisting of at least three carbon atoms which is capable of forming a detectable complex with a polypeptide when the compound is incorporated into a double-stranded ribonucleotide acid, deoxyribonucleic acid duplex, or DNA-RNA hybrid;

wherein the dotted line represents a linkage or group joining B and A, provided that if B is purine, the linkage is attached to the 8-position of the purine, if B is 7-deazapurine, the linkage is attached to the 7-position of the deazapurine, and if B is pyrimidine, the linkage is attached to the 5-position of the pyrimidine; and

wherein each of x, y and z represents

[formula 2]

(4) A kit in accordance with claim 1, wherein said chemically-labeled nucleotide has the formula,

[formula 3]

wherein each of B, B' and B" represents a purine, deazapurine, or pyrimidine moiety covalently bonded to the $C^{1'}$-position of the sugar moiety, provided that whenever B, B' or B" is purine or deazapurine, it is attached at the N^9-position of the purine or deazapurine, and whenever B, B' or B" is pyrimidine, it is attached at the N^1-position;

wherein A represents a moiety consisting of at least three carbon atoms which is capable of forming a detectable complex with a polypeptide when the compound is incorporated into a double-stranded duplex formed with a complementary ribonucleic or deoxyribonucleic acid molecule;

wherein the dotted line represents a chemical linkage or group joining B and A, provided that if B is purine, the linkage is attached to the 8-position of thepurine, if B is 7-deazapurine, the linkage is attached to the 7-position of the deazapurine,

and if B is pyrimidine, the linkage is attached to the 5-position of the pyrimidine;

wherein Z represents H- or HO-; and

wherein m and n represent integers from 0 up to about 100,000.

(5) A kit in accordance with claim 1 wherein said substrate contains said double-stranded polynucleotide attached thereto, said double-stranded polynucleotide comprising said single-stranded non-radioactive chemically labeled polynucleotide or nucleotide, the other strand being unlabled single-stranded polynucleotide, said substrate being prepared by fixing said other single-stranded unlabeled polynucleotide to said substrate and then hybridizing to said unlabled polynucleotide said one strand containing said chemically labeled polynucleotide or nucleotide.

(6) A kit in accordance with claim 1 wherein said single-stranded non-radioactive chemically labeld polynucleotide contains at least 25 bases which are substantially complementary to the bases making up the other unlabeled single-stranded polynucleotide of said double stranded polynucleotide.

(7) A kit in accordance with claim 1 wherein said chemically labeled polynucleotide is a polynucleotide coupled to or attached to a polypeptide.

(8) A kit in accordance with claim 1 wherein said chemically labeled polynucleotide is a polynucleotide coupled to or attached to a polypeptide, said polypeptide being terminally ligated to said polynucleotide.

(9) A kit in accordance with claim 1 wherein said chemically labeled polynucleotide comprises or has attached thereto an amino acid or polypeptide comprising a Sig chemical moiety covalently attached therto, said Sig chemical moiety being capable of signalling itself or making itself self-detecting or its presence known.

(10) A kit in accordance with claim 9 wherein said Sig chemical moiety comprises a saccharide component.

(11) A kit in accordance with claim 9 wherein said Sig chemical moiety includes a co-enzyme.

(12) A kit in accordance with claim 11 wherein said co-enzyme is selected from the group consisting of thiamine pyrophosphate, flavine mononucleotide, flavine adenine dinucleotide, nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, co-enzyme A, pyridoxyl phosphate, biotin, tetrahydrofolic acid, coenzyme B_{12}, lipoic acid and ascorbic acid.

(13) A kit in accordance with claim 1 wherein said chemically labeled polynucleotide comprises or has attached thereto a monosaccharide or a polysaccharide comprising a Sig chemical moiety attached thereto, said Sig chemical moiety being capable of signalling itself or making itself self-protecting or its presence known.

(14) A kit in accordance with claim 13 wherein said Sig chemical moiety comprises a chelating agent.

(15) A kit in accordance with claim 13 wherein said Sig chemical moiety includes a co-enzyme.

(16) A kit in accordance with claim 15 wherein said co-enzyme is selected from the group consisting of thiamine pyrophosphate, flavine mononucleotide, flavine adenine dinucleotide, nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, co-enzyme A, pyridoxyl phosphate, biotin, tetrahydrofolic acid, coenzyme B_{12}, lipoic acid and ascorbic acid.

(17) A kit in accordance with claim 1 wherein said double-stranded polynucleotide is a double-stranded polyribonucleotide.

(18) A kit in accordance with claim 1 wherein said double-stranded polynucleotide is a double-stranded polydeoxyribonucleotide.

(19) A kit in accordance with claim 1 wherein said double-stranded polynucleotide comprises as one of the strands polydeoxyribonucleotide and the other strand a polyribonucleotide.

(20) A kit in accordance with claim 1 wherein said substrate is transparent and wherein said substrate is adapted for the transmission of light therethrough for color observation or colorimetric determination of said double-stranded polynucleotide on said transparent substrate.

(21) A kit in accordance with claim 20 wherein said transparent substrate is glass.

(22) A kit in accordance with claim 1 wherein said substrate is translucent and wherein said substrate is adapted for the transmission of light therethrough for color observation or colorimetric determination of said double-stranded polynucleotide on said transparent substrate.

(23) A kit in accordance with claim 1 wherein said substrate is a planar substrate provided with a well therein wherein said double-stranded polynucleotide is fixed.

(24) A kit in accordance with claim 23 wherein said substrate carrying said double-stranded polynucleotide fixed to a well therein is adapted for the transmission of light therethrough for the photometric or colorimetric determination of the double-stranded polynucleotide fixed within said well.

(25) A kit in accordance with claim 1 wherein said substrate is a plastic-coated substrate.

(26) A kit in accordance with claim 1 wherein said substrate is a glass-coated substrate.

(27) A kit in accordance with claim 1 wherein said substrate is a plastic substrate.

(28) A kit in accordance with claim 1 wherein said substrate is a

polystyrene substrate.

(29) A kit in accordance with claim 1 wherein said substrate is a polyethylene substrate.

(30) A kit in accordance with claim 1 wherein said substrate is a polypropylene substrate.

(31) A kit in accordance with claim 1 wherein said substrate is a celluose substrate.

(32) A kit in accordance with claim 1 wherein said substrate is a nitrocellulose substrate.

(33) A kit in accordance with claim 1 wherein said substrate is a dextran substrate.

(34) A kit in accordance with claim 1 wherein said substrate is an epoxy or expoxy-coated substrate.

(35) A kit in accordance with claim 1 wherein said substrate has amino groups attached or fixed to the surface thereof.

(36) A kit in accordance with claim 1 wherein said substrate provides a surface capable of fixing or immobilizing negatively charges polyelectroytes thereon.

(37) A kit in accordance with claim 1 wherein said substrate provides a surface capable per se or untreated of fixing or immobilizing single-stranded or double-stranded polynucleotides thereto or permits the hybridization of a non-radioactive chemically-labeled polynucleotide to an unlabeled single-stranded polynucleotide or DNA fixed to said surface.

(38) A kit in accordance with claim 1 wherein said substrate is a planar form transparent glass plate provided with rows of wells or depressions formed on the surface thereof, the surface of said wells or depressions having fixed thereto double-stranded polynucleotide, one of the strands of said double-stranded nucleotide being a non-radioactively chemically-labeled

polynucleotide or comprises a non-radioactively chemically-labeled nucleotide as a nucleotide component of said one strand.

(39) A kit in accordance with claim 1 wherein said substrate is a cuvette having a planar surface adapted for the transmission of light therethrough.

(40) A kit in accordance with claim 1 wherein said substrate is adapted for the transmission of light perpendicularly through the substrate.

(41) A kit in accordance with claim 1 wherein said substrate is adapted for the transmission of light transversely through the system.

(42) A kit in accordance with claim 1, wherein said substrate is a transparent glass container or cuvette provided with planar wells, the interior surface of the container having fixed thereto double-stranded polynucleotide, one of the strands of said double-stranded polynucleotide being a non-radioactively chemically-labeled polynucleotide or comprises a non-radioactively chemically-labeled nucleotide as a nucleotide component of said one-strand.

(43) A kit in accordance with claim 1, wherein said substrate has a glass surface and said glass surface is treated by a method for fixing genetic DNA material thereto which comprises treating the glass surface with aqueous nitric acid solution at a temperature of about the boiling point of the nitic acid solution, washing the resulting nitric acid-treated glass surface and, after drying the thus-treated glass surface, contacting the thus-treated glass surface with gamma-aminopropyltriethoxysilane, washing and drying the resulting treated glass surface and fixing DNA material thereto.

(44) A kit in accordance with claim 1, wherein said substrate has a transparent to glass surface and said glass surface suitable for fixing genetic DNA material thereto, said glass surface having attached or fixed thereto amino groups.

(45) A kit in accordance with claim 1, further comprising:

a plurality of porous or non-porous substrates, a double stranded oligonucleotide or polynucleotide being directly or indirectly fixed to each substrate,

wherein one of the strands comprises one or more non-radioactive chemically-labeled nucleotides or one or more chemical labels which comprises a signal moiety or moieties which are capable of generating a soluble signal.

(46) A kit in accordance with claim 1, further comprising :

a plurality of transparent non-porous or translucent non-porous systems capable of retaining or containing a fluid or solution,

in each system a double stranded oligonucleotide or polynucleotide is directly or indirectly fixed or immobilized to a substrate,

wherein one of the strands comprises one or more non-radioactive chemically-labeled nucleotides or one or more chemical labels which comprises a signal moiety or moieties which are capable of generating a soluble signal.

(47) A kit in accordance with claim 46, wherein said system is selected from the group consisitng of a well, a tube, a cuvette and an apparatus which comprises a plurality of said wells, tubes or cuvettes.

(48) A kit in accordance with claim 46, wherein the device is capable of or adapted for measuring a soluble signal by a means selected from the group consisting of photometric techniques, spectrophotometric techniques, enzyme-linked immunosorbent assay techniques, colorimetric techniques, chemiluminescent techniques, fluorometric techniques and immunofluorometric techniques.

(49) A kit in accordance with claim 1, further comprising an array of nucleotide acid strands which comprises different and known sequences covalently fixed or immobilized to said substrate.

(50) A method for determining the presence of a selected genetic material which comprises treating a sample containing said selected genetic material so as to denature the same to provide a single strand thereof derived from said genetic material, fixing the resulting single strand of genetic material to a surface, contacting under hybridizing conditions the thus-fixed single strand of genetic material with a non-radioactively chemically-labeled polynucleotide probe substantially complementary to the nucleotide components characterizing said genetic material to be determined and detecting duplex formation between said single strand of genetic material and said non-radioactive chemically-labeled polynucleotide probe by contacting said probe with fluid or solution to generate a soluble signal which is dissolved and diffused in said fluid or solution.

(51) A method in accordance with claim 50 wherein said surface is a transparent surface.

(52) A method in accordance with claim 50 wherein said surface is a translucent surface.

(53) A method in accordance with claim 50 wherein said surface is a glass surface.

(54) A method in accordance with claim 50 wherein said surface is a glass surface and wherein said single-stranded genetic material is fixed to a well provided on said surface, said surface being adapted for the transmission of light therethrough for the photometric or colorimetric determination of duplex formation between said single-stranded genetic material and said polynucleotide probe.

(55) A method in accordance with claim 50 wherein said duplex formation between said single-strand of genetic material and said non-radioactive, chemically-labeled polynucleotide probe

is detected or determined by enzyme linked immunosorbent assay (ELISA).

(56) A method in accordance with claim 50 wherein detection of duplex formation by the said single-strand of genetic material and said non-radioactive, chemically-labeled polynucleotide probe is determined by forming a complex with a chemical label providing on said polynucleotide probe, said complex comprising a component capable of signalling or eliciting its presence by spectrophotometric or colorimetric means.

(57) A method in accordance with claim 50 wherein detection of duplex formation between said single-strand of genetic material and said non-radioactive, chemically-labeled polynucleotide probe is determined by forming a complex with a chemical label provided on said polynucleotide probe, said complex comprising an enzyme, and eliciting the presence of said enzyme-containing complex attached to said chemical label by bringing said enzyme-containing complex attached to said polynucleotide probe into contact with a substrate which undergoes a chemical or color change when in contact with said enzyme.

(58) A method in accordance with claim 50 wherein duplex or double strand formation or hybridization between said single strand of material and said non-radioactive chemically-labeled polynucleotide probe is determined by providing said chemical label attached to said polyncleotide probe, before or after duplex formation, with a chelating agent and eliciting the presence of said chelating agent by contacting said chelating agent with a substrate which undergoes a chemical reaction or color change when in contact with said chelation agent.

(59) A method in accordance with claim 58 wherein eliciting the presence of said chelating agent is carried out by contacting said chelating agent with a substrate which undergoes a chemical reaction when in contact with said chelating agent.

(60) A method in accordance with claim 58 wherein eliciting the presence of said chelating agent is carried out by contacting said chelating agent with a substrate which undergoes a color

change when in contact with said chelating agent.

(61) A method in accordance with claim 60 wherein said color change is photometrically or colorimetrically determined and is indicative of the amount of chelating agent fixed to the resulting formed duplex.

(62) A method for detecting the presence of a pathogen in a clinical sample suspected of containing said pathogen, said method comprising deposition said sample on an innert transparent or translucent support, treating said sample to affix genetic material of any of said pathogens present in said sample to said support in substantially single-stranded form, contacting said fixed single-strandedgenetic material with a non-radioactive chemically-labeled probe having a nucleotide sequence of at least about 25 bases at least substantially complementary to a nucleotide sequence of a structural gene characteristic of said pathogen, said chemically labeled probe having therein a chemically labeled nucleotide in accordance with claim 3, said contacting being underhybridizing conditions at a predetermined stringency and detecting duplex formation on said support by contacting said probe with fluid or solution to generate a soluble signal which is dissolved and diffused in said fluid or solution.

(63) A method in accordance with claim 62 wherein said chemically labeled nucleotide has the formula,

[formula 3]

wherein each of B, B' and B" represents a purine, deazapurine, or pyrimidine moiety covalently bonded to the $C^{1'}$-position of the sugar moiety, provided that whenever B, B' or B" is purine or deazapurine, it is attached at the N^9-position of the purine or deazapurine, and whenever B, B' or B" is pyrimidine, it is attached at the N^1-position;

wherein A represents a moiety consisting of at least three carbon atoms which is capable of forming a detectable complex with a polypeptide when the compound is incorporated into a

double-stranded duplex formed with a complementary ribonucleic or deoxyribonucleic acid molecule;

wherein the dotted line represents a chemical linkage or group joining B and A, provided that if B is purine, the linkage is attached to the 8-position of thepurine, if B is 7-deazapurine, the linkage is attached to the 7-position of the deazapurine, and if B is pyrimidine, the linkage is attached to the 5-position of the pyrimidine;

wherein Z represents H- or HO-; and

wherein m and n represent integers from 0 up to about 100,000.

(64) A method in accordance with claim 62 wherein said pathogen is selected from the group consisting of a streptococcus, a staphylococcus, a pneumococcus, a meningococcus, a salmonolla typhimurium and a clostridium botulinum.

(65) A method for detecting the presence or identity of genetic DNA material which comprises depositing a sample of genetic material on an inert transparent or transleucent support surface, treating said sample to affix said genetic DNA material to said support substantially single-stranded form, contacting said fixed single-stranded genetic DNA material with a non-radioactive chemically-labeled probe having a nucleotide sequence of at least about 25 bases, at least substantially complementary to the nucleotide sequence contained in said genetic DNA material, said chemically-labeled probe having therein a chemically-labeled nucleotide in accordane with claim 3, said contacting being under hybridizing conditions at a predetermined sringency and detecting duplex formation of said genetic DNA material fixed to said support surface by means of said chemically-labeled probe.

(66) A device for detecting or measuring a color change or chemical reaction colorimetrically or photometrically which comprises;

a substrate which has associated with or fixed thereto a

single stranded chemically labeled DNA probe hybridized to a complementary single stranded DNA material, said chemical label having an enzyme complex attached thereto,

means for adding a chemical substrate to contact said enzymecomplex attached to said chemical label of said hybridized single stranded DNA probe associated with or fixed to said substrate in a transparent non-porous or translucent non-porous system so as to generate by reaction between said enzyme complex and said chemical substrate a color change or a photometrically detectable chemical reaction,

means for illuminating or passing light onto or through said substrate containing said enzyme complex involved in said color change or chemical reaction as a soluble signal dissolved and diffused in a fluid or solution comprising means for colorimetrically or photometrically detecting said color change or photometrically detectable chemical reaction brought about by contact between said enzyme complex and said chemical substrate.

(67) A device in accordance with claim 66 wherein said means for illuminating or passing light onto or through said substrate containing said enzyme complex involved in said color change or said chemical reaction includes means for quantitatively measuring said color change or said photometrically detectable chemical reaction.

(68) A device in accordance with claim 66, wherein said chemically labeled polynucleotide comprises the formula in accordance with claim 9.

(69) A device in accordance with claim 66, wherein said chemically labeled polynucleotide comprises the formula in accordance with claim 3.